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14. ABSTRACT

MS is a common cause of neurological disability. Though there are several effective treatments for the relapsing-remitting form of the disease, many patients either present with primary progressive MS, or eventually progress to secondary progressive MS, for which there are no treatment options. Though pathologically all MS patients have oligodendroglia injury and apoptosis, the pathologic hallmark of *progressive MS* is axon damage and neuron loss. We have recently characterized a novel function of oligodendroglia-- metabolic support of axons by supplying lactate thru the MCT1 transporter-- and hypothesize that loss of, or injury to, oligodendroglia in MS may produce axon damage through a failure of energy supply via loss of function of this critical oligodendroglial protein. The successful completion of the Aims in this grant will not only further our understanding of axon degeneration in MS, but also provide a potential novel treatment strategy for patients with progressive MS. We hypothesize that reduced expression of MCT1 in injured oligodendroglia of multiple sclerosis patients contributes to axon neurodegeneration and that increasing MCT1 will be protective in the progressive forms of the disease. From a short-term perspective, these studies will provide the foundation of research necessary to determine if this pathway is a viable approach to consider for protecting axons and preventing the chronic neuronal injury that underlies progressive MS. In the long term, independent of this proposal- new innovative approaches toward enhancing this oligodendroglial pathway may provide one day – a powerful means to alter long-term neurodegeneration in MS and cortical and motor disability that accompanies neuronal axonal damage in MS.

15. SUBJECT TERMS
MS, MCT1, metabolic support, axon injury, myelin, therapy, oligodendroglia

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Table of Contents

Hypothesis: Reduced expression of MCT1 in injured oligodendroglia of multiple sclerosis patients contributes to axon neurodegeneration and that increasing MCT1 will be protective in the progressive forms of the disease.

Specific Aims:

Aim 1: Determine if oligodendroglial MCT1 expression is significantly altered in human autopsy samples and animal models of MS. *Hypothesis: MCT1 is downregulated in injured oligodendroglia in both MS patients and MS animal models.*

1.1 MCT1 expression by Western blot in grey and white matter cortices from MS patients

1.2 MCT1 expression by Western blot, real-time RT PCR and MCT1 BAC reporter in acute diptheria toxin deletion of oligodendroglia, cuprizone, and experimental autoimmune encephalomyelitis (EAE) mouse models

Aim 2. **Determine if the loss of MCT1 exacerbates axonal and neuronal injury in experimental MS models.** *Hypothesis: Downregulation of MCT1 will exacerbate the axonal injury in MS animal models.*

2.1 Conditional MCT1 knockout selectively within oligodendroglia in EAE and cuprizone models

2.2 Focal knockdown of MCT1 by AAV-MCT1 shRNA in spinal cord or corpus callosum in EAE and cuprizone models.

Aim 3. Determine if increased expression of MCT1 mitigates the injury to CNS axons in experimental MS models. *Hypothesis: Upregulation of MCT1 in MS animal models will ameliorate the axonal injury.*

3.1 Conditional MCT1 overexpresion within oligodendroglia in EAE and cuprizone models

3.2 Focal upregulation of MCT1 by AAV-MCT1 construct in spinal cord or corpus callosum in EAE and cuprizone models

A. **Research Progress.**

Progress Aim 1: Determine if oligodendroglial MCT1 expression is significantly altered in human autopsy samples and animal models of MS.

1) Human MS

We acquired human tissue from MS patients and controls from Dr. Peter Calebresi (Johns Hopkins University) and plan to acquire additional MS and control samples from Dr. Bruce Trapp (Cleveland Clinic). We were recently able to successfully extract myelin from fresh post mortem CNS tissue samples in order to look specifically at oligodendrocyte MCT1 dysregulation in MS patients. *We now have early data that there is a loss of MCT1 in MS white matter (Fig 1).* We plan to continue to quantify expression of MCT1 and other oligodendroglia proteins (i.e., myelin basic protein (MBP) and CNPase) by Western blots from grey and white matter autopsy samples from MS patients and control patients.

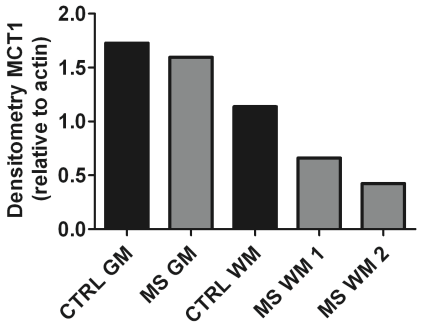

Fig 1. *MCT1 Western Blot of Human MS Brain*. MCT1 in white matter (WM) of multiple sclerosis (MS) brain is decreased compared to control (CTRL)white matter. No change seen in MCT1 in MS grey matter (GM) compared to CTRL GM.

2 Mouse models of MS

In collaboration with the laboratory of Peter Calabresi we started looking at MCT1 dysregulation at both the mRNA and protein level in animal models of MS. In a first set of experiments we performed EAE on MCT1tdTomato reporter mice, ie mice that harbour a tdTomato reporter driven by the MCT1 promoter and are reflective of active mRNA transcription. These mice were backcrossed into the C57Bl6/J strain for at least 10 generations (which took more than a year to accomplish). EAE was induced as described previously (Way, Popko et al, Nat. Commun. 2015) at the age of 8 weeks and mice were euthanized 22 days later. In order to identify MCT1tdT expressing oligodendrocytes, we co-labeled MCT1tdT with Olig2, a well known pan oligodendrocyte-lineage marker, expressed by immature oligodendrocyte progenitor cells (OPCs) and a subset of mature oligodendrocytes.

As expected, we found that in the EAE treated animals, there was a significant decrease in the number of Olig2+MCT1tdT+ cells in the EAE treated mice as compared to controls (Fig 2, n=3-4, $p<0.05$).

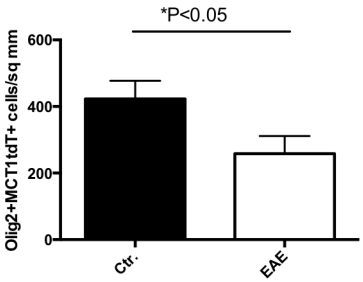

Fig 2. Quantification of MCT1tdT+Olig2+ oligodendrocytes in spinal cord white matter of EAE induced mice relative to controls.

We also looked at MCT1 protein using a MCT1 chicken antibody generated in our laboratory and previously found to be specific for MCT1 (data not shown). MCT1 protein expression could not be detected in oligodendrocytes in either control or EAE mice, as MCT1 protein in oligodendrocytes is thought to be mildly expressed in specific oligodendrocyte compartments like the paranodal and/or juxtaparanodal region (KA Nave, personal communication), two regions adjacent to the node of Ranvier. Interestingly and unexpectedly, we found that MCT1 protein, mildly expressed by GLT-1 expressing astrocyte processes in control mice, was significantly upregulated in GLT-1 expressing astrocytes in regions affected by EAE, notably in the spinal cord dorsal horn white matter (Fig 3). In addition, some MCT1tdT (RNA reporter) expression was co-localized with GLT-1, a mature astrocyte marker, which could be reflective of either astrocyte MCT1 expression, or phagocytosis of MCT1tdT containing material by astrocytes (Fig 3, lower panels).

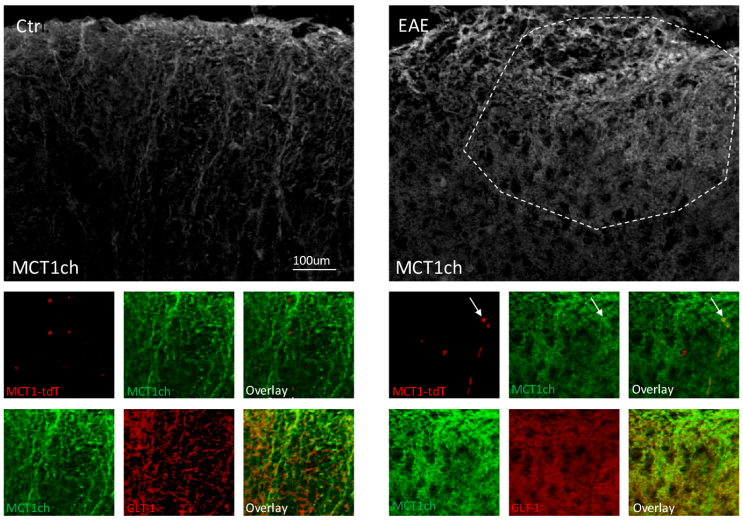

Fig 3. MCT1 protein immunostaining (MCT1ch) in controls and mice induced with EAE. In the EAE group, the dashed line delineates a region with clear MCT1 protein upregulation after EAE induction. In the EAE mice, we found rare MCT1tdT (MCT1 mRNA reporter) co-localizing with MCT1chicken antibody (arrows). There was a strong upregulation of MCT1ch in EAE, which strongly co-localizes with GLT-1, a marker for mature astrocytes. The MCT1tdT reporter expression found co-localizing with MCT1 protein is indicative of either astrocyte MCT1 upregulation or phagocytosis of MCT1tdT containing debris by MCT1 expressing astrocytes.

In a second set of experiments we started looking at MCT1 in the Multiple sclerosis –like cuprizone mouse model. In this model, MCT1tdTomato mice were backcrossed to the C57Bl6/J strain for >10 generations and 7 week old mice were treated with 0.2% cuprizone for 6 weeks. Mice were analysed two weeks after cessation of cuprizone treatment, a period during which extensive remyelination is occurring in this mouse model. Looking at MCT1tdT reporter expression, no significant changes in reporter expression were detected between treated group versus control, probably reflective of the ongoing re-generation of newly generated oligodendrocytes that goes along with oligodendrocyte injury during the treatment period (Fig 4, middle panel). This data also indicates that repopulating oligodendrocytes derived from OPCs retain the ability to initiate MCT1 reporter expression despite a very reactive microenvironment (reactive astrocytes and microglia, data not shown).
On the other hand, remyelination was still incomplete at this time point as shown by the reduced MBP expression in the cuprizone treated group. (Fig 4, top panel). We are currently analyzing whether MCT1 protein is affected in the corpus callosum by performing myelin preparations of the cuprizone treated tissue. As we did previously for the EAE mouse model, we also performed immunostaining with our MCT1 chicken antibody. There was a significant increase in MCT1 protein expression most notable in the corpus callosum (Fig 4, lower panel) as well as in striatal nerve bundles (data not shown). In the corpus callosum MCT1 protein clearly co-

localized with GLT-1 expressing astrocytes, very similar to what was found in the EAE experiments (see above), suggesting that astrocytes respond to the demyelinating insult by an upregulation of MCT1 protein.

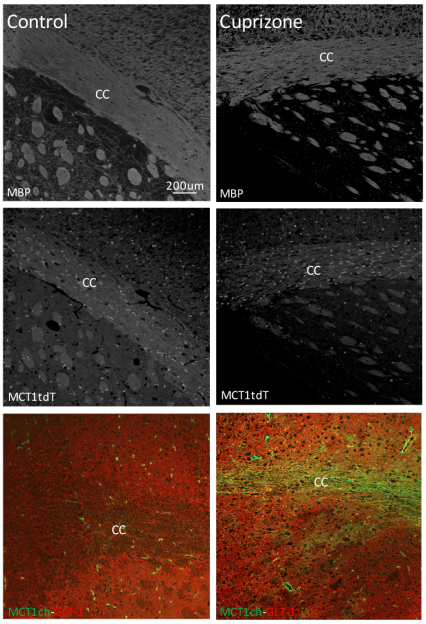

Fig 4. MCT1 reporter (RNA) and protein expression in the cuprizone mouse model of demyelination. Two weeks after cessation of a 6 week cuprizone treatment there was a slight loss of MBP expression (top panel) but no loss of MCT1tdT reporter positive oligodendrocytes (middle panel) in the corpus callosum (CC) of the cuprizone treated group as compared to controls. When using an antibody directed against MCT1 (MCT1ch, green, lower panel) we found a dramatic upregulation of MCT1 protein in the corpus callosum (CC) of cuprizone treated mice compared to controls. This MCT1 upregulation extensively co-localized with GLT-1, the mature astrocyte marker.

<u>Progress Aims 2 and Aim 3:</u>

<u>Aim 2</u>. Determine if the loss of MCT1 exacerbates axonal and neuronal injury in experimental MS models.

For this aim we have thus far focussed on generating MCT1 conditional null mice and crossing these mice with oligodendrocyte and astrocyte specific Cre drivers. These mouse experiments took longer than expected to complete due to the extensive genetic crossing required and subsequent validation of the genetic crossings. As can be seen in Fig 5 oligodendrocyte specific knockout of MCT1 using the MogiCre and PLPCreER lines led to a significant reduction in MCT1 protein in myelin preparations derived from whole spinal cord (Fig 5, n=3, $p<0.001$ and $p<0.01$ resp.). In addition, we also crossed our MCT1 conditional mice with GFAP-Cre mice which drives Cre expression selectively in astrocytes. We hypothesize that blocking MCT1 in astrocytes would lead to either significant myelin debris accumulation and/or axonal degeneration as oligodendrocytes and astrocytes are unable to provide neurons with trophic support. Using this mouse line we also obtained a strong reduction of MCT1 protein expression in whole spinal cord extracts. The remaining MCT1 protein expression in conditional knockout is reflective of MCT1 protein in other cell types like oligodendrocytes and endothelial cells.

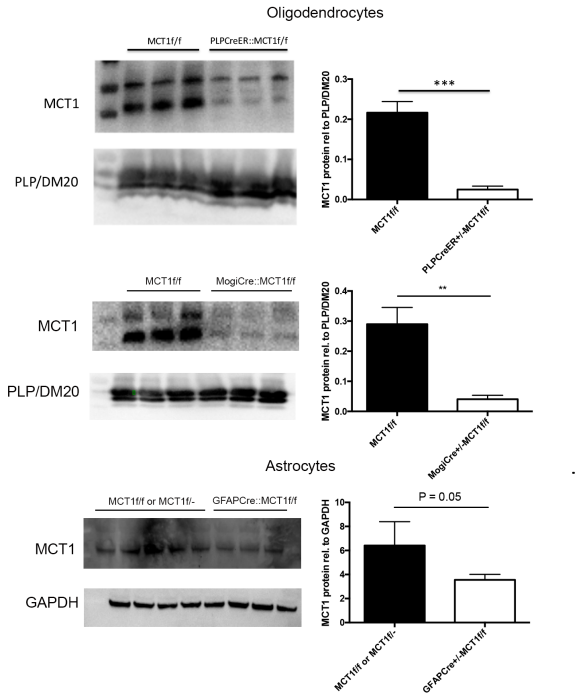

Fig 5. Loss of MCT1 protein when MCT1 conditional knockout mice are crossed with oligodendrocyte or astrocyte specific Cre lines. (top panel) After cossing with the MogiCre line, we obtained around 90% reduction in MCT1 expression in myelin extracts at P60. (middle panel) After crossing with the tamoxifen inducible PLPCreER mouse, we obtained around 90% reduction in MCT1 protein expression in myelin extracts (3,5 months after tamoxifen injection in P100 old mice). (lower panel) After crossing with the GFAP-Cre line, we obtained a near significant reduction in MCT1 protein in lumbar spinal cord protein extracts at P30.

<u>Aim 3</u>. Determine if increased expression of MCT1 mitigates the injury to CNS axons in experimental MS models.

For this aim we recently obtained a viral vector overexpressing MCT1. Our collaborator Thomas McCown at Univ of North Carolina, generated an adeno-associated viral (AAV) vector containing a construct expressing MCT1 fused to GFP under control of the MBP promoter. This AAV was injected in P0 newborn mouse pups and mice were analyzed 7 weeks later. We obtained a strong GFP signal in the corpus callosum and in white matter lumbar spinal cord regions (Fig 6). We are currently exploring the level of MCT1 expression obtained in corpus callosum and spinal cord. This AAV will be injected in P0 mouse C57Bl6/J mice

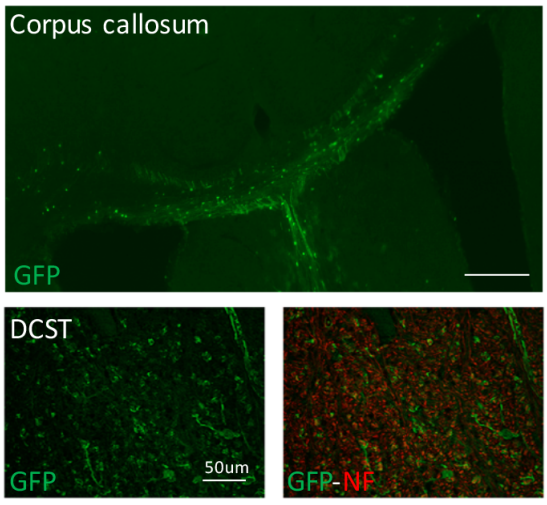

Fig 6. The AAV-MBP-GFP vector was intracerebroventricularly (ICV) injected at P0 C57Bl6/J mice. GFP expression, driven by the MBP promotor in oligodendrocytes, was assessed 7 weeks after injection. We found profound GFP expression in corpus callosum and in spinal cord white matter regions (DCST = dorsocorticospinal tract)(the latter co-labeled with neurofilament (NF) in red).

Key Research Accomplishments/Conclusions/Impact

So far we have found compelling evidence that astrocytes respond in MS (and other) models of demyelination by a significant upregulation of MCT1 protein expression. This was unexpected- and demonstrates the great value of the various reporter mice and the conditional deletion mice we have taken time to generate. We are

currently trying to figure out the nature of this upregulation. One hypothesis is that astrocytes upregulate MCT1 to compensate for the loss of oligodendrocytes (and the oligodendroglia MCT1) who lose their capacity to provide metabolic support to the neurons. Alternatively, astrocytes specifically respond to demyelination by phagocytosis of myelin debris, as has been shown to occur previously, at least in vitro, but presumably also in mouse models of MS (Gaultier, personal communication). The MCT1tdT reporter 'expression' in astrocytes as shown in the EAE mouse model (Fig 3) could be indicative of myelin debris phagocytosis: ie residual oligodendrocyte derived MCT1tdT accumulating in astrocytes. This would increase the astrocyte metabolic demand and enhance glucose conversion to lactate leading to a lactate build up in astrocytes. The MCT1 upregulation would ensure that lactate does not accumulate (leading to cell death due to lactate acidosis), but rather would escape to the extracellular environment where other cells (remyelinating oligodendrocytes and neurons) could re-use lactate as an oxidative metabolite. It is interesting to note that a similar MCT1 upregulation was found in different models of dys –and demyelination in our studies: eg the Shiverer mouse model as well as the ALS mutant SOD1 mouse (our unpublished observations). All these models are also characterized by the astrocyte upregulation of low density lipoprotein receptor-related protein 1 'LRP1', a presumptive phagocytosis receptor, well characterized as being involved in a-beta clearance in models of Alzheimer's disease, providing more evidence for a potential role of astrocytes in myelin phagocytosis in models of demyelination. The upregulation of MCT1 protein in astrocytes did not occur in models of specific neuronal ablation, hinting that this astrocyte response is not a mere aspecific response to neuroinjury but rather specific for models of demyelination and a specific reaction to oligodendrocyte injury. The exact nature of the factor(s) expressed by oligodendrocytes that induce thus astrocyte response are still unknown.

As a consequence of these new findings we would propose to study the role of astrocyte MCT1 in addition to oligodendrocyte MCT1 in the next year. To begin to address this new and relevant biology, we have already crossbred GFAP-Cre mice with our MCT1 conditional knockout mice. In these mice, MCT1 can be specifically depleted from GFAP expressing astrocytes (Fig 5) during the disease course of EAE/cuprizone treatment. These experiments might shed light on a compelling metabolic role of astrocytes in response to demyelination/remyelination, more specifically the astrocyte influence on the surrounding oligodendrocytes and neurons.

Reportable Outcomes: None

References: None

Appendices: None